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ORIGINAL ARTICLE

# Anti-Inflammatory of *Alhagi sparsifolia* Shap. Extract: Network Pharmacology Analysis and Experimental Verification

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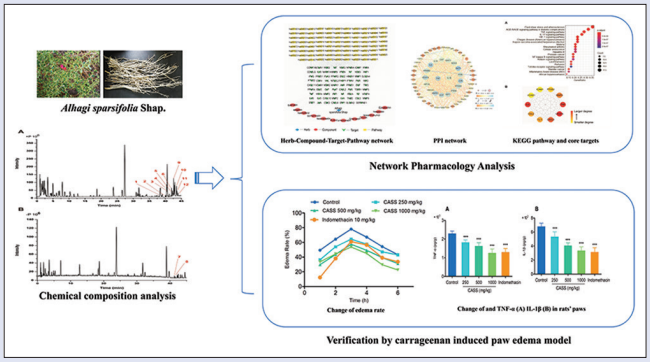
## ABSTRACT

**Background:** *Alhagi sparsifolia* Shap. (ASS) is a treasured medicinal plant and has long been employed for treating human diseases including physiological process of pain and inflammation in China. However, very tiny information has been stated about active ingredients and action mechanisms of ASS. **Objective:** The main determination of this article is to examine analgesic and anti-inflammatory properties of chloroform fraction of *Alhagi sparsifolia* Shap. (CASS), wishing to deliver some basic data for further growth of new drugs for the treatment of inflammation and pain. **Materials and Methods:** The acute toxicity of CASS was assessed. Essential compositions of CASS were entreated by ultra-performance liquid chromatography/tandem mass spectrometry. Network pharmacology analysis comprising Herb-Chemical component-Targets-Pathway (H-C-T-P) network conducting and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis were performed. The anti-inflammatory property of CASS and regulation of two core targets prophesied by network pharmacology were confirmed by a carrageenan-induced paw edema model. **Results:** CASS was safe even at the maximum oral administration dose of 20 g/kg. Twelve active ingredients were preliminary recognized by ultra-performance liquid chromatography/tandem mass spectrometry. Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were found to be the core targets projected by H-C-T-P network. By oral administration, CASS could suggestively relieve carrageenan-induced edema and downregulate TNF-α and IL-1 β concentration in rats' swelling paw since dose of 250 mg/kg. **Conclusion:** CASS has anti-inflammatory effect through complex mechanisms which encompass downregulating the activation of IL-1 β and TNF-α.

**Key words:** *Alhagi sparsifolia* Shap., carrageenan-induced paw edema, inflammatory, interleukin-1β, network pharmacology, tumor necrosis factor-α

## SUMMARY

- *Alhagi sparsifolia* Shap. has long been employed for treating human diseases including physiological process of pain and inflammation in China. By network pharmacology analysis associated experimental verification, this article presented the proof that chloroform fraction of *Alhagi sparsifolia* Shap. acted its anti-inflammatory effect major through flavonoid components by inhibiting the increase of IL-1β and TNF-α.




**Abbreviations Used:** ASS: *Alhagi sparsifolia* Shap.; CASS: chloroform fraction of *Alhagi sparsifolia* Shap.; H-C-T-P: Herb-Chemical component-Targets-Pathway; UPLC-MS/MS: ultra-performance liquid chromatography-tandem mass spectrometry; ESI: RT: Retention time; Electrospray ionization; KEGG: Kyoto Encyclopedia of Genes and Genomes; DAVID: Database for Annotation, Visualization and Integrated Discovery; TTD: Therapeutic Target Database; PPI: protein-protein interaction; SD: Sprague Dawley; TNF-α: Tumor Necrosis Factor-Alpha; IL-1β: Interleukin-1β

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## INTRODUCTION

Patients suffering from chronic pain mandatory long-term use of non-steroidal anti-inflammatory drugs, which often hints to serious side effect.[1] Herbal medicine, considered by low poisonous effect and multitarget mechanisms, delivers another way for the search of new anti-inflammatory drugs and has concerned much more consideration in recent years.[2,3] *Alhagi*, one member of the genus *Papilionaceae* subfamily in *Leguminosae* family, comprises seven species around the world. Three of the seven (*Alhagi pseudalhagi, Alhagi sparsifolia,* and *Alhagi maurorum*) are dispersed in lowland saline meadows of Inner Mongolia, Xinjiang, Gansu, and Ningxia desert region of China.[4] As logged in flora of China, *A. sparsifolia* Shap. (ASS) is a grassy perennial herb that grasps up to 50–100 cm with red flowers and linear pulses. It has been employed as folk medicine ever since Qing dynasty. Sugar grain secreted by ASS was first documented in "Ben Cao Gang Mu Shi Yi" (Supplements to compendium of material, published in 1765 A. D.). Different parts of ASS have been used in folk recipes for different human



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ailments. The leaves are employed in tumor and joint gall therapy, the flowers are used for heat-clearing and detoxication, the seeds are used for toothache, and the whole plant are reported to cure enteritis.[5] The most extensive use of ASS is in treatment of rheumatic arthritis and abdominal pain of diarrhea. It is not firm to find that the complaints mentioned above are all related to anti-inflammatory activities. However, the experimental studies on the anti-inflammatory effect of ASS and the related mechanism have not been established. Based on the result of our earlier studies, chloroform extract of ASS (CASS) displayed better anti-inflammatory activity than ethyl acetate extracts and petroleum ether extracts. This article was intended to explore anti-inflammatory properties of CASS, furthermore, wishing to deliver some basic data for further development of new drugs for the treatment of inflammation.

## MATERIALS AND METHODS

ASS was gained from Xinjiang Uygur Autonomous Region in September, 2014 and identified by Professor Yang (Shaanxi Academy of Traditional Chinese Medicine, Xi'an, China). A voucher specimen (140919) was conserved in the medicinal material herbarium at Shaanxi Academy of Traditional Chinese Medicine. CASS was organized from ASS by our laboratory.

Carrageenan was obtained from Sigma-Aldrich (C1013, St. Louis, MO, USA). Enzyme-linked immunosorbent assay (ELISA) kits for mouse tumor necrosis factor-α (TNF-α) and IL-1β were obtained from the Hysen Hung Industrial Co., Ltd. Shanghai (PR China). Indomethacin tablet was subscribed from YiKang Pharmaceutical Supermarket at Xi-hua-men Road, Xi'an, Shaanxi province; ethyl alcohol, chloroform, ethyl acetate, and petroleum ether were acquired from Sinopharm Chemical Reagent Shaanxi Co., Ltd (PR China). The experimental water was distilled water arranged by our laboratory.

ICR mice (20.0 ± 2.0 g) and SD rats (200 ± 20 g) were procured from Laboratory Animal Breeding and Research Centre of Xi'an Jiao tong University. The Animal Care and Use Committee of the Shaanxi Academy of Traditional Chinese Medicine permitted the use of animals. All the experiments in this work were conducted according to the guidelines delivered by the Chinese Food and Drug Administration and Animal Research: Reporting *in vivo* Experiments.

### Preparation of chloroform extract of *Alhagi sparsifolia* Shap.

The whole plant was dried naturally and crushed. 10 kg of coarse powder was accomplished with reflux extraction of 75% ethanol (V/V) for three times (2 h/time). Mixed extracting solution was determined under reduced pressure at 40°C, then dried at 60°C. The ethanol extract powder was suspended in water and orderly extracted by petroleum, ethyl acetate, and chloroform. After vacuum concentration, a total of 2.85 g CASS was acquired with yield of 0.49%.

### Acute toxicity evaluation of chloroform extract of *Alhagi sparsifolia* Shap.

In the pretest, CASS at 20 g/kg, 2.0 g/kg, and 0.2 g/kg doses were pragmatic to three groups of ICR mice, respectively. After 7-day reflection, no acute toxic reaction had been found. Afterward, maximum toleration test was carried out with 20 mice at the maximum dose (20 g/kg).[6]

### Chemical composition analysis of chloroform extract of *Alhagi sparsifolia* Shap.

Essential compositions of CASS were discovered by ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS). Thermo QE focus system equipped with electrospray ionization source (ESI), and TraceFinder working station was engaged. CASS, eluted in 70% ethanol (v/v) to concentration of 50 mg/mL, was analyzed using a Hypersil GOLD $C_{18}$ column (150 mm × 2.1 mm, 2.6 um) at 30°C. Sixty percent A (0.1% (v/v) formic acid water) and 40% B ($CH_3CN$) were used as mobile phase, 0.30 mL/minute as elution rate, 2 μL as injection volume. Isocratic elution program was as follows: 0 min, 60% A; 0–0.1 min, 60% A; 0.1–35 min, 20% A; 35–40 min, 10% A; 40–45 min, 10% A.

As for mass analysis condition, nebulizer ($N^2$) was employed as atomized and auxiliary gas, 3.0 kV as voltage in negative ion mode, 0.3 mL/min as flow rate, 300°C and 400°C as the ion source and curtain gas temperature, 20 ~ 50 eV as the collision energy.

The chemical profiles of CASS were analyzed by UPLC-MS/MS. All UPLC-MS data, comprising retention times, accurate molecular masses, and MS/MS data are essential for the structural analysis of compounds. The element compositions were calculated and clearly established by combining with a mass accuracy (ppm) <5.0 using TraceFinder 3.0 software (Thermo Fisher, Waltham, MA, USA). Identification of the composition of CASS is based on comparison of fragment ion information to the standard MS/MS spectra from the database MassBank (https://massbank.eu/), references from the literature and the obtainable authentic standards.

### Network pharmacology analysis of chloroform extract of *Alhagi sparsifolia* Shap.

Targets of active compounds were composed by TCMSP database. Targets related to inflammation were unruffled through TCMSP, OMIM, and Therapeutic Target Database (TTD) database. The inflammation-related target interactions were collected from STRING database and imported into Cytoscape 3.7.0 (Cytoscape Consortium, San Diego, CA, USA) to build chemical composition-related targets protein-protein interaction (PPI) and inflammation targets PPI. By using merge function in Cytoscape, the extraction of above two PPI networks, a quick Herb-Compound-Target (H-C-T) network was produced. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was carried out on the targets in H-C-T network using Database for Annotation, Visualization and Integrated Discovery (DAVID) database. Finally, H-C-T pathway (H-C-T-P) network was created, and network extension heterogeneous scrutiny was carried out.[7-11]

### Pharmacological verification on anti-inflammatory activities of chloroform extract of *Alhagi sparsifolia* Shap.by carrageenan induced paw edema model

Carrageenan-induced paw edema model was used to confirm the anti-inflammatory activities of CASS. Sprague Dawley (SD) rats (200 ± 20 g) were acquired from Laboratory Animal Breeding and Research Centre of Xi'an Jiao tong University. Eligible mice were accustomed for 7 days before experiments, preserved under standard laboratory conditions (22 ± 2°C with relative humidity 50 ± 15%, 12 h light and dark photoperiod), and fed with standard rodent pellet diet.

Forty-eight healthy male rats were squarely and randomly alienated into control group, model group, positive control group, and three treatment groups. In the first 1 week, the rats in the positive control group and three treatment groups were treated with indomethacin (0.01 g/kg), CASS (0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg) once a day, respectively. At the 7$^{th}$ day, 2 h after the last administration, the carrageenan-induced paw edema test was accompanied.[12-14]

## Determination of cytokine levels

At the end of the carrageenan model, the rats were forfeited. The hind paws of the rats were detached and sheared ground, immerged in 4 mL of normal saline for 6 h at 4°C, and centrifuged at 4000 rpm for 15 min to get the supernatant. The content of TNF-α and IL-1 β was spotted by mouse ELISA kits[15] in accordance with the manufacturer's instructions.

## Statistical analysis

Data were examined using IBM SPSS 19.0 (SPSS, Chicago, IL, USA). One-way ANOVA was employed for intergroup comparisons; $P < 0.05$ was deliberated statistically significant.

# RESULTS

## Acute toxicity evaluation of chloroform extract of *Alhagi sparsifolia* Shap.

No death happened at the maximum tolerance dose of 20 g/kg, no acute toxicity symptoms related was witnessed, and signifying that oral administration of CASS was safe.

## Chemical composition of chloroform extract of *Alhagi sparsifolia* Shap.

The base peak ion chromatogram of CASS was shown in Figure 1 [Figure 1-a denotes positive ion model and b denotes negative ion model]. By comparison with standard or references, a total of twelve compounds were recognized from CASS in the ESI + and ESI − modes. The mass spectrometry and references information were planned in Table 1.

## Herb-compound-target-pathway network of chloroform extract of *Alhagi sparsifolia* Shap.

Targets related to the 12 chemicals in CASS were composed. Inflammation-related targets were encompassed 153 target proteins and were transferred into Official Gene Symbol through UniProt database. The active ingredient-action target network was attained by extracting the intersection of constituent-target network and acute inflammation-related targets set through merge function of Cytoscape 3.7.0. H-C-T-P network was revealed in Figure 2. Network extension analysis obtained 170 nodes and 28,730 constituent-target action relationship. The average number of targets per compound was 9.859, which means a very thick network.

## The PPI network analysis and prediction of core targets

As Figure 3 presented, targets of larger degree, which incorporated TNF, AKT1, MAPK1, EGF, JUN, IL6, PTGS2 and so on, were put in the inner circle to signify more important targets.

KEGG pathway enrichment analysis on the ingredient-related targets was performed using DAVID database. The top 20 pathways were itemized in Figure 4a. TNF signaling pathway and IL-17 signaling pathway, located at the 3 and 4 position, were the two pathways that are powerfully connected with cytokines.

Thus, it is reasonable to forecast that the regulation of cytokines may play a key role in the treatment of acute inflammation by CASS. To further discover the key targets in the core action pathway, we designed a new set composed of targets involved in the top 5 pathways. After another functional PPI analysis of this core set, an interesting consequence appeared, cytokines of TNF and IL-1 β were very core targets involved in the top 5 pathways [Figure 4b]. Based on the above analysis results, it is reasonable to speculate that TNF and IL-1 β may be key cytokines regulating acute inflammation in CASS.

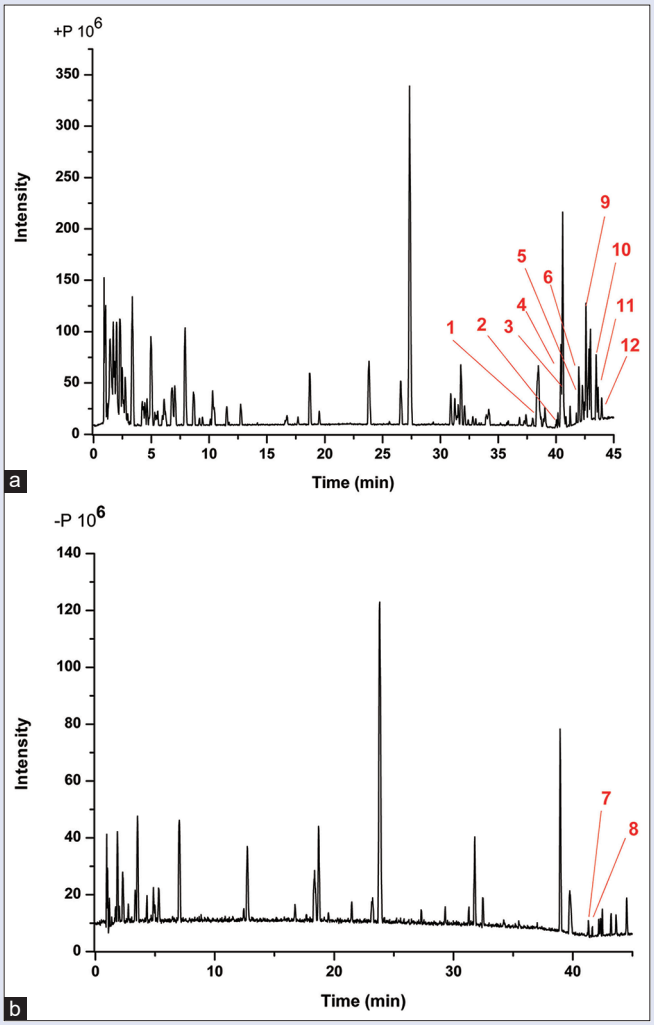


**Figure 1:** The base peak ion current chromatogram of chloroform fraction of *Alhagi pseudalhagi* Shap. in electrospray ionization source + (a) and electrospray ionization source– (b) modes

## Pharmacological verification on anti-inflammatory activities of chloroform extract of *Alhagi sparsifolia* Shap. by carrageenan-induced paw edema model

Comparison of rats' right paw volume in unlike times was shown in Table 2. It is clear that the right paw swelling in the rats was substantial in all experiment groups from 1 h onward to 6$^{th}$ h. Edema caused by carrageenan injection is a biphasic event. The first phase (90–180 min) is in concern with the histamine, serotonin, bradykinin, *etc.*[16] The second phase (270–360 min) is connected to the activation of prostaglandins and lysosome.[17] As revealed in Figure 5, paw swelling persisted for all over 6 h and the maximum percentage of 78.08% in control group emerged at 3 h after injection. Different doses of CASS exhibited inhibition rate at different level. In general, CASS at dose of 500 mg/kg and 1000 mg/kg displays greater inhibition ability than the dose of 250

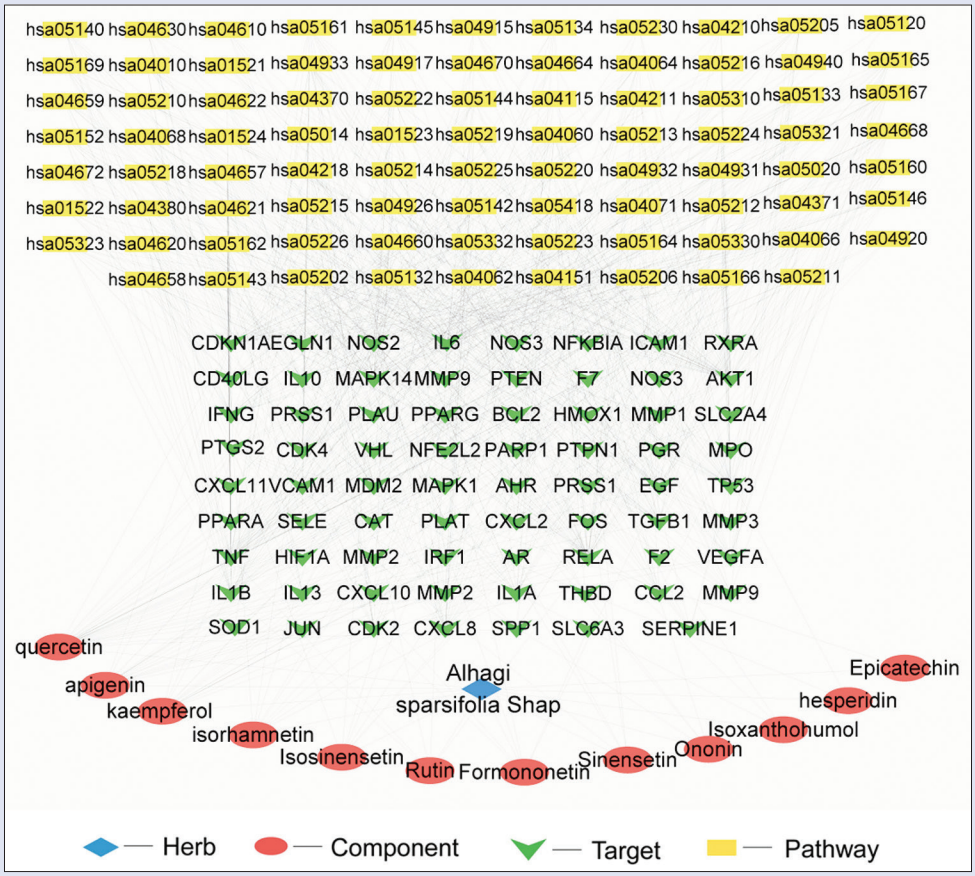


**Figure 2:** Herb-compound-target-pathway network of chloroform fraction of *Alhagi pseudalhagi* Shap.

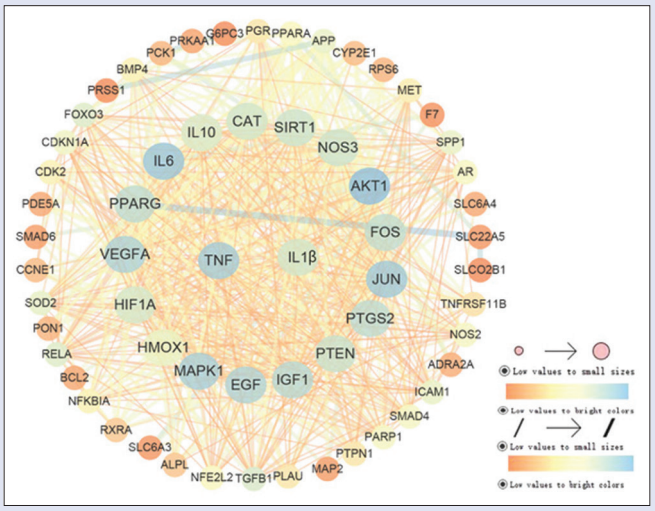


**Figure 3:** PPI network of the active ingredient-related targets

mg/kg. In the time range of 3 h to 6 h, dose of 1000 mg/kg even exhibited better activity than indomethacin. More intuitive proof of inhibition rate exposed in Table 3. In the earlier stage of the test, indomethacin presented the most excellent activity with the inhibition rate reached 74.96%, while CASS at the dose of 1000 mg/kg indicated a much longer activity in the whole test, with the inhibition rate keeping over 40% even to the 6 h postinjection. In this regard, CASS has a benefit over the positive medicine since it can play more lifelong anti-inflammation effect than indomethacin.

## The inhibition of chloroform extract of *Alhagi sparsifolia* Shap. on tumor necrosis factor-α, interleukin -1β in swollen feet of acute arthritis rats

As shown in Figure 6, indomethacin at 10 mg/kg presented noteworthy inhibition of IL-1β and TNF-α production. Oral administration of CASS (250, 500, and 1000 mg/kg) formed substantial reduction of IL-1 β and TNF-α ($P < 0.001$) in rats' paw sections. For different doses, CASS disclosed diverse inhibition ability.

# DISCUSSION

The main chemical constituents in CASS analyzed by UPLC-MS/MS were frequently flavonoids. Some components also have been described in other *Alhagi* species.[18,19] It is worth to remark that the other kind of *Alhagi* species, *A. maurorum*, was also testified to relieve neuroinflammation.[20-22] Since *A. maurorum* and ASS both belong to *Alhagi*, the common ingredients may contribute to anti-inflammatory pharmacological activity in all the two herbs. Among the components, quercetin has been conveyed to act anti-inflammatory property

**Table 1:** Putatively identified 12 constituents in chloroform fraction of *Alhagi pseudalhagi* Shap.

| Putative identification | ESI mode | RT (min) | Formula | m/z estimated | Major MS/MS fragments | References |
|---|---|---|---|---|---|---|
| Epicatechin | Positive | 38.49 | $C_{15}H_{14}O_6$ | 291.09 | 165.05<br>151.04<br>139.04<br>123.04 | MassBank database |
| Rutin | Positive | 40.21 | $C_{27}H_{30}O_{16}$ | 611.15 | 225.64 | MassBank database |
| Hesperidin | Positive | 40.92 | $C_{28}H_{34}O_{15}$ | 611.19 | 303.09 | MassBank database |
| Quercetin | Positive | 40.96 | $C_{15}H_{10}O_7$ | 447.09 | 304.28<br>107.21 | [38] |
| Ononin | Positive | 41.67 | $C_{22}H_{22}O_9$ | 431.30 | 270.20 | MassBank database |
| Apigenin | Positive | 41.85 | $C_{15}H_{10}O_5$ | 271.06 | 135.02<br>99.06<br>85.03 | MassBank database |
| Kaempferol | Negative | 41.98 | $C_{15}H_{10}O_6$ | 285.04 | 267.03<br>239.05 | MassBank database |
| Isoxanthohumol | Negative | 42.08 | $C_{21}H_{22}O_5$ | 353.14 | 233.08<br>119.05 | MassBank database |
| isorhamnetin | Positive | 42.47 | $C_{16}H_{12}O_7$ | 317.07 | 256.04<br>201.06 | MassBank database |
| Isosinensetin | Positive | 43.45 | $C_{20}H_{20}O_7$ | 373.13 | 358.10 | [39] |
| Sinensetin | Positive | 43.98 | $C_{20}H_{20}O_7$ | 373.12 | 343.22<br>269.08 | [40] |
| Formononetin | Positive | 44.75 | $C_{16}H_{12}O_4$ | 269.08 | 254.05<br>237.05<br>197.05 | MassBank database |

Identification is based on the listed references and comparison with standard mass spectrometry in MassBank database. CASS: Chloroform fraction of *Alhagi pseudalhagi* Shap.; ESI: Electrospray ionization; MS/MS: Tandem mass spectrometry; RT: Retention time

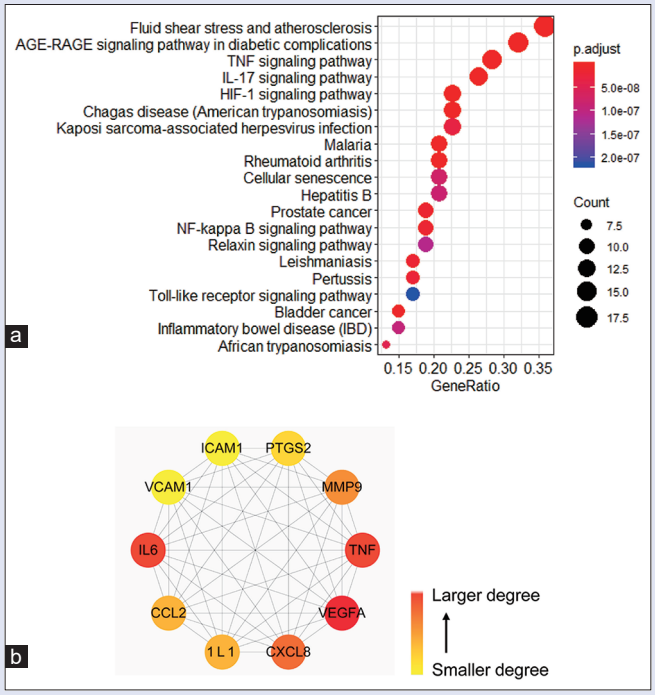


**Figure 4:** Kyoto Encyclopedia of Genes and Genomes pathway (a) and core targets in the top five Kyoto Encyclopedia of Genes and Genomes pathways (b)

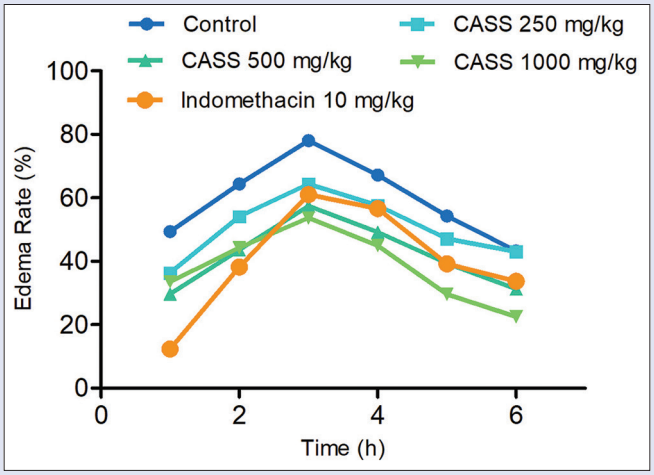


**Figure 5:** Edema rate of rats in carrageenan-induced paw edema test

through downregulation of ICAM-1 and MMP-9 in TNF-α-activated retinal pigment epithelia cells.[23,24] Besides, isorhamnetin has also been demonstrated to downregulate some key molecules such as TNF-α, COX-2, $PGE_2$, and nuclear factor-κB convoluted in inflammation.[25,26] In addition, formononetin and pratensein existing anti-inflammatory activity that can be allied with their antioxidant properties and/or their interactions with inflammatory mediators.[27-29] Epicatechin was evidenced to produce a substantial reduction in plasma PGE (2) TNF, IL-1 β, and IL-6 in the carrageenan-induced paw edema model.[30] Rutin,[31] ononin,[32] kaempferol,[33] and apigenin[34] were all recounted to be related to anti-inflammatory effects. These anti-inflammatory activity reports of these components deliver reference suggestion of anti-inflammatory activity for CASS.

Network pharmacology method is a prevalent method established in recent years applying to evaluate mechanism of plant or herbal medicine. It can combine multidisciplinary technologies such as computer science, chemistry, pharmacology, pharmacokinetics together to build chemical composition-targets-disease-pharmacological action network and can filter system of herbal medicine treatment

**Table 2:** Comparison of rats' right paw volume in different times

| Time (h) | Paw volume (mL) | | | | |
|---|---|---|---|---|---|
| | Control | CASS (250 mg/kg) | CASS (500 mg/kg) | CASS (1000 mg/kg) | Indometacin (10 mg/kg) |
| 0 | 1.323±0.056 | 1.221±0.031 | 1.563±0.082 | 1.486±0.110 | 1.258±0.084 |
| 1 | 1.976±0.043*** | 1.665±0.033*** | 2.028±0.052*** | 1.984±0.108*** | 1.414±0.102* |
| 2 | 2.175±0.072*** | 1.882±0.139*** | 2.246±0.110*** | 2.144±0.096*** | 1.739±0.111*** |
| 3 | 2.356±0.069*** | 2.008±0.110*** | 2.462±0.134*** | 2.284±0.132*** | 2.026±0.077*** |
| 4 | 2.212±0.039*** | 1.925±0.040*** | 2.332±0.135*** | 2.154±0.073*** | 1.971±0.109*** |
| 5 | 2.042±0.040*** | 1.797±0.087*** | 2.179±0.131*** | 1.926±0.032*** | 1.752±0.093*** |
| 6 | 1.895±0.042*** | 1.727±0.088*** | 2.052±0.101*** | 1.821±0.024*** | 1.682±0.097*** |

*$P$<0.05; ***$P$<0.001 compared with 0 h. CASS: Chloroform fraction of *Alhagi pseudalhagi* Shap.

**Table 3:** Inhibition rate of chloroform fraction of *Alhagi pseudalhagi* Shap. and indometacin in carrageenan-induced paw edema test

| Time (h) | Inhibition rate (%) | | | | |
|---|---|---|---|---|---|
| | Control | CASS (250 mg/kg) | CASS (500 mg/kg) | CASS (1000 mg/kg) | Indometacin (10 mg/kg) |
| 1 | - | 26.37 | 39.78 | 32.07 | 74.96 |
| 2 | - | 15.95 | 32.2 | 31.23 | 40.64 |
| 3 | - | 17.54 | 26.37 | 31.21 | 21.83 |
| 4 | - | 14.25 | 26.81 | 33.10 | 15.76 |
| 5 | - | 13.23 | 27.48 | 45.43 | 27.74 |
| 6 | - | 0.45 | 27.69 | 47.85 | 22.14 |

CASS: Chloroform fraction of *Alhagi pseudalhagi* Shap.

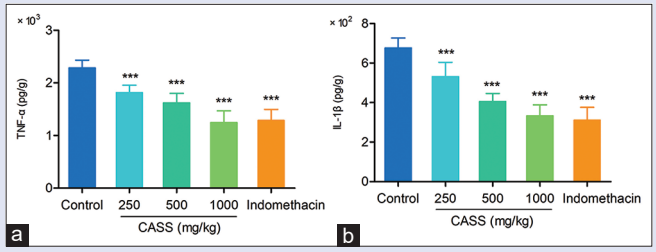


**Figure 6:** Levels of and tumor necrosis factor -α; (a) interleukin -1β; (b) in rats' experimental paw

of a disease specific chemical composition, possible targets and related signaling network, so as to disclose the characteristics of the interaction between drug and target network. In this article, the network pharmacology analysis based on the 12 components recognized from CASS was conducted and delivered theoretical basis for the mechanism prediction of CASS for the first time. The core targets analyzed by network topologies such as TNF, VEGFA, IL-1 β, AKT1, and MAPK1 have been the emphasis of many literatures. Among these targets, TNF and IL-1 β have caught our consideration. According to the data of H-C-T-P, TNF and IL-1 β are the core targets linked more active ingredient and budding pathways than most other targets. PPI analysis delivered more intuitive evidence: TNF and IL-1 β were in the core targets set which share more relationships with other imperative targets.

In addition, abundant studies have stated the imperative function of pro-inflammatory cytokines (such as IL-1 β and TNF-α) in initiation and progression of acute and chronic inflammation. TNF-α is a multifunctional pro-inflammatory cytokine, playing a pivotal role in the genesis of inflammatory mechanical hyper nociception in rats and the nociceptive response in mice. It can persuade the expression of adhesion factors in synovial tissues and then affect inflammatory responses.[35] IL-1β is serious to the pathogenesis of a change of pathological process by regulating inflammation, angiogenesis, hematopoiesis, and cognition through the IL-1 β pathway.[36]

Based on the above analysis, the anti-inflammatory activity and the potential core targets of CASS have been anticipated. There is adequately of information pointing to two core targets: IL-1 β and TNF-α. Thus, pharmacodynamics validation of CASS against acute inflammation was performed. The carrageenan-induced paw edema test is an extremely reproducible model for anti-inflammatory drug screening. Hind paw edema of rats caused by carrageenan injection is a biphasic event. The first phase (90–180 min) is in consequence with histamine, serotonin, bradykinin, *etc*.[16] The later phase (270–360 min) is related to the activation of prostaglandins and lysosome[17] and so on. According to the results of carrageenan-induced paw edema test, CASS presented a dose-dependent anti-inflammation activity at the dose range of 250 mg/kg to 1000 mg/kg in both the two phases. Exclusively, inhibition rate of 1000 mg/kg dose surpassed indomethacin for 3 h. In this regard, CASS has benefits as it can play a more lifelong anti-inflammation effect than indomethacin.

As for cytokine levels purpose, in carrageenan-induced paw edema test, carrageenan persuades the production of TNF-α,[37] which activates IL-1β production. Pro-inflammatory cytokines of IL-1β and TNF-α in swollen joint of rats were determined by ELISA kits. The results established that CASS, oral administration at 250 mg/kg to 1000 mg/kg, is able to constrain the production of TNF-α and IL-1 β in rats swelling paws, which can preliminary authorize the role of CASS in the regulation of core targets TNF-α and IL-1 β. While, more work also needs to be done for further exploration of the material foundation and action mechanism of CASS.

## CONCLUSION

CASS has anti-inflammatory effect through complex mechanisms which encompass downregulating the activation of IL-1 β and TNF-α. The results in this article deliver resultant directions for forthcoming research, we will further examine the active ingredients and potential action mechanism of CASS, more complete exploration needs to be done in the future.

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Nil.

## Conflicts of interest

There is no conflict of interest.

## REFERENCES

1. Abadi SS, Moin A, Veerabhadrappa GH. Review article: Fabricated microparticles: An innovative method to minimize the side effects of NSAIDs in arthritis. Crit Rev Ther Drug Carrier Syst 2016;33:433-88.
2. Yatoo MI, Gopalakrishnan A, Saxena A, Parray OR, Tufani NA, Chakraborty S, *et al.* Anti-inflammatory drugs and herbs with special emphasis on herbal medicines for countering inflammatory diseases and disorders – A review. Recent Pat Inflamm Allergy Drug Discov 2018;12:39-58.
3. Tsai TC, Tung YT, Kuo YH, Liao JW, Tsai HC, Chong KY, *et al.* Anti-inflammatory effects of *Antrodia camphorata*, a herbal medicine, in a mouse skin ischemia model. J Ethnopharmacol 2015;159:113-21.
4. Jin ZH, Xu KH. Chemical composition and clinical application of *Alhagi sparsifol*. Jilin Tradit Chin Med 2009;29:711-2.
5. Yuan ZY, He XJ, Huang PY. Research status and progress on *Alhagi sparsifolia*. J Anhui Agri Sci 2012;40:9674-7.
6. Xu SY, Bian RL, Chen X. Experimental Methodology of Pharmacology. Beijing: People' Medical Publishment House; 2002. p. 201-10.
7. Liang Y, Zhang X, Zou J, Shi Y, Wang Y, Tai J, *et al.* Pharmacology mechanism of *Flos magnoliae* and *Centipeda minima* for treating allergic rhinitis based on pharmacology network. Drug Dev Ind Pharm 2019;45:1547-55.
8. Zhao RL, He YM. Network pharmacology analysis of the anti-cancer pharmacological mechanisms of *Ganoderma lucidum* extract with experimental support using Hepa1-6-bearing C57 BL/6 mice. J Ethnopharmacol 2018;210:287-95.
9. Liu XW, Tang CL, Zheng H, Wu JX, Wu F, Mo YY, *et al.* Investigation of the hepatoprotective effect of Corydalis saxicola Bunting on carbon tetrachloride-induced liver fibrosis in rats by $^{1}$H-NMR-based metabonomics and network pharmacology approaches. J Pharm Biomed Anal 2018;159:252-61.
10. Lee AY, Park W, Kang TW, Cha MH, Chun JM. Network pharmacology-based prediction of active compounds and molecular targets in Yijin-Tang acting on hyperlipidaemia and atherosclerosis. J Ethnopharmacol 2018;221:151-9.
11. Shu Z, He W, Shahen M, Guo Z, Shu J, Wu T, *et al.* Clarifying of the potential mechanism of Sinisan formula for treatment of chronic hepatitis by systems pharmacology method. Biomed Pharmacother 2018;100:532-50.
12. Samuel OB, Lawrence SB. Ethanolic leaf extract from Strophanthus gratus (Hook.) Franch. (Apocynaceae) exhibits anti-inflammatory and antioxidant activities. Cogent Biology 2019;5:1-11.
13. Winter CA, Risley EA, Nuss GW. Anti-inflammatory and antipyretic activities of indomethacin, 1- (p-chlorobenzoyl) -5- methoxy-2- methyl-indole -3- acetic acid. J Pharm Exp Ther 1963;141:369-76.
14. Moon SM, Lee SA, Hong JH, Kim JS, Kim DK, Kim CS. Oleamide suppresses inflammatory responses in LPS-induced RAW264.7 murine macrophages and alleviates paw edema in a carrageenan-induced inflammatory rat model. Int Immunopharmacol 2018;56:179-85.
15. El-Tedawy DM, Abd-Alhaseeb MM, Helmy MW, Ghoneim AI. Systemic bee venom exerts anti-arthritic and anti-inflammatory properties in a rat model of arthritis. Biomed Rep 2020;13:20.
16. Crunkhorn P, Meacock SC. Mediators of the inflammation induced in the rat paw by carrageenin. Br J Pharmacol 1971;42:392-402.
17. Thomazzi SM, Silva CB, Silveira DC, Vasconcellos CL, Lira AF, Cambui EV, *et al.* Antinociceptive and anti-inflammatory activities of Bowdichia virgilioides (sucupira). J Ethnopharmacol 2010;127:451-6.
18. Sultan A, Moohammadnor M, Eshbakova KA. Chemical constituents of *Alhagi pseudalhagi*. Chem Natur Compounds 2011;47:140-1.
19. Alimova DF, Eshbakova KA, Bobakulov KM. A new flavonol glycoside from the aerial part of *Alhagi pseudalhagi*. Chem Natur Compounds 2013;49:437-9.
20. Awaad AS, El-meligy RM, Qenawy SA, Atta AH, Soliman GA. Anti-inflammatory, antinociceptive and antipyretic effects of some desert plants. J Saudi Chem Soc 2011;15:367-73.
21. Zhou D, Wei H, Jiang Z, Li X, Jiao K, Jia X, *et al.* Natural potential neuroinflammatory inhibitors from *Alhagi sparsifolia* Shap. Bioorg Med Chem Lett 2017;27:973-8.
22. Atta AH, Abo EL-Sooud K. The antinociceptive effect of some Egyptian medicinal plant extracts. J Ethnopharmacol 2004;95:235-8.
23. Hou DD, Zhang W, Gao YL, Sun YZ, Wang HX, Qi RQ, *et al.* Anti-inflammatory effects of quercetin in a mouse model of MC903-induced atopic dermatitis. Int Immunopharmacol 2019;74:1-11.
24. Cheng SC, Wu YH, Huang WC, Pang JS, Huang TH, Cheng CY. Anti-inflammatory property of quercetin through downregulation of ICAM-1 and MMP-9 in TNF-α-activated retinal pigment epithelial cells. Cytokine 2019;116:48-60.
25. Lu X, Liu T, Chen K, Xia Y, Dai W, Xu S, *et al.* Isorhamnetin: A hepatoprotective flavonoid inhibits apoptosis and autophagy via P38/PPAR-α pathway in mice. Biomed Pharmacother 2018;103:800-11.
26. Shi H, He J, Li X, Han J, Wu R, Wang D, *et al.* Isorhamnetin, the active constituent of a Chinese herb *Hippophae rhamnoides* L, is a potent suppressor of dendritic-cell maturation and trafficking. Int Immunopharmacol 2018;55:216-22.
27. El-Bakoush A, Olajide OA. Formononetin inhibits neuroinflammation and increases estrogen receptor beta (ERβ) protein expression in BV2 microglia. Int Immunopharmacol 2018;61:325-37.
28. Wang J, Wang L, Zhou J, Qin A, Chen Z. The protective effect of formononetin on cognitive impairment in streptozotocin (STZ)-induced diabetic mice. Biomed Pharmacother 2018;106:1250-7.
29. Liang C, Tan S, Huang Q, Lin J, Lu Z, Lin X. Pratensein ameliorates β-amyloid-induced cognitive impairment in rats via reducing oxidative damage and restoring synapse and BDNF levels. Neurosci Lett 2015;592:48-53.
30. Al-Sayed E, Abdel-Daim MM. Analgesic and anti-inflammatory activities of epicatechin gallate from *Bauhinia hookeri*. Drug Dev Res 2018;79:157-64.
31. Liu S, Adewole D, Yu L, Sid V, Wang B, O K, *et al.* Rutin attenuates inflammatory responses induced by lipopolysaccharide in an *in vitro* mouse muscle cell (C2C12) model. Poult Sci 2019;98:2756-64.
32. Dong L, Yin L, Zhang Y, Fu X, Lu J. Anti-inflammatory effects of ononin on lipopolysaccharide-stimulated RAW 264.7 cells. Mol Immunol 2017;83:46-51.
33. Yeon MJ, Lee MH, Kim DH, Yang JY, Woo HJ, Kwon HJ, *et al.* Anti-inflammatory effects of kaempferol on *Helicobacter pylori*-induced inflammation. Biosci Biotechnol Biochem 2019;83:166-73.
34. Charalabopoulos A, Davakis S, Lambropoulou M, Papalois A, Simopoulos C, Tsaroucha A. Apigenin exerts anti-inflammatory effects in an experimental model of acute pancreatitis by down-regulating TNF-α. *In Vivo* 2019;33:1133-41.
35. Shan MQ, Qian Y, Yu S, Guo SC, Zhang L, Ding AW, *et al.* Anti-inflammatory effect of volatile oil from *Schizonepeta tenuifolia* on carrageenin-induced pleurisy in rats and its application to study of appropriate harvesting time coupled with multi-attribute comprehensive index method. J Ethnopharmacol 2016;194:580-6.
36. Navarro SA, Serafim KG, Mizokami SS, Hohmann MS, Casagrande R, Verri WA Jr. Analgesic activity of piracetam: Effect on cytokine production and oxidative stress. Pharmacol Biochem Behav 2013;105:183-92.
37. de-Almeida SC, da-Silva ÂC, Sousa NR, Amorim IH, Leite BG, Neves KR, *et al.* Antinociceptive and anti-inflammatory activities of a triterpene-rich fraction from *Himatanthus drasticus*. Braz J Med Biol Res 2019;52:1-2.
38. Girme A, Saste G, Chinchansure A, Joshi S, Kunkulol R, Hingorani L, et al. Metabolic profiling and *in vivo* hepatoprotective activity of Malpighia glabra L. leaves. J Food Biochem. 2021; 45:1-15.
39. Kim H, Moon JY, Mosaddik A, Cho SK. Induction of apoptosis in human cervical carcinoma HeLa cells by polymethoxylated flavone-rich Citrus grandis Osbeck (Dangyuja) leaf extract. Food and Chemical Toxicology 2010; 45:2345-2442.
40. Ke ZL, Yang Y, Tan S, Zhou ZQ. Characterization of Polymethoxylated Flavonoids in the Peels of Chinese Wild Mandarin (Citrus reticulata Blanco) by UPLC-Q-TOF-MS/MS. Food Anal. Methods. 2017; 10:1328-38.